

## LEARNED IMMOBILITY IS ALSO INVOLVED IN THE FORCED SWIMMING TEST IN MICE

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A modified version of the forced swimming test (FST) was utilised in order to test, for the first time in mice, the learned immobility hypothesis. From this point of view, the subjects learn to be immobile in the first session, being the second one a retention test. The development of habituation was observed by repeating the test. The forgetting was studied by allowing different time intervals between the first and the second session. A decrease in the activity was observed with intervals of up to 18 days, but not with longer intervals of 21 or 24 days. Scopolamine (1 or 2 mg/kg), a cholinergic antagonist, did not modify the swimming activity in the second session. Physostigmine, a cholinergic agonist, at a dose of 0.05 mg/kg was ineffective, and at a dose of 0.2 mg/kg decreased the swimming activity in the second session. These data extend to mice the findings previously obtained in rats, and lend additional support to the learned immobility hypothesis in the interpretation of the behaviour found in the FST.

*La Inmovilidad Aprendida está también Implicada en la Prueba de Natación Forzada en Ratones.* Se utilizó una versión modificada de la prueba de natación forzada (P.N.F.) para poner a prueba, por primera vez en ratones, la hipótesis de la inmovilidad aprendida. Desde este punto de vista, los sujetos aprenden a estar inmóviles en la primera sesión, siendo la segunda un test de retención. Se observó el desarrollo de habituación a través de la repetición de la prueba. Se estudió el olvido utilizando diferentes intervalos de tiempo entre la primera y la segunda sesión. La actividad descendió con intervalos de 18 días o menores, pero no con los intervalos de 21 y 24 días. La escopolamina (1 o 2 mg/kg), un antagonista colinérgico, no modificó la actividad natatoria en la segunda sesión. La fisostigmina, un agonista colinérgico, no fue efectiva con una dosis de 0.05 mg/kg, pero sí lo fue con una dosis de 0.2 mg/kg; la actividad natatoria disminuyó en la segunda sesión. Estos datos extienden a ratones los hallazgos previamente obtenidos en ratas y dan apoyo a la hipótesis de la inmovilidad aprendida a la hora de interpretar la conducta en la P.N.F.

The forced swimming test (FST) is commonly used for the assessment of the anti-

depressant-like properties of drugs (Cesana, Ciprandi and Borsini, 1995; Cohen, Perrault and Sanger, 1997; Hemby et al., 1997). In the original Porsolt test (Porsolt, Le Pichon and Jalfre, 1977b), rats are immersed during a fifteen minute pre-test in a cylindrical tank with water. Twenty four hours later, the rats are replaced in the cylinder for five minutes

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and the total duration of immobility is measured. In mice (Porsolt, Bertin and Jalfre, 1977a), the procedure is similar except that the animals are immersed in water for six min (test session) without a pre-test session. The initial vigorous activity gives way to a so called «immobility posture». The duration of immobility, in both types of rodents, is recorded by a trained observer. This behaviour is regarded as an animal analogue of depression and in fact, antidepressant compounds tend to reduce the time spent by the animals in immobility.

Since quite a number of antidepressants decrease immobility in this animal model, its predictive validity is well established (Willner, 1984, 1991). Nevertheless, numerous substances are «false positives» and in spite of decreasing immobility they do not possess antidepressant properties in humans. These include: anticholinergics (Bhattacharya and Sen, 1991; Browne, 1979), nootropics (Schmidt, 1984), opiates and opioids (Ben Natan, Chaillet, Lecomte, Marcais, Uchida and Costentin, 1984). Even anisomycin, an antibiotic not tested as antidepressant in humans and not expected to function as such from the present understanding of the disorder, gives «positive» in the FST (De Pablo, Parra, Segovia and Guíllamón, 1989). With respect to face validity (phenomenological similarity between the model and the modelled disorder) and construct validity (theoretical rationale for the model), it can be said that the FST has some small degree of the former and nothing of the latter (Willner, 1984).

A different interpretation of the FST holds that rats, in this behavioural paradigm, learn to be immobile. Immobility is considered to be an adaptive response to the situation consisting of animals learning to keep their heads out of water with a minimum of energy expenditure. The germinal idea was introduced by Hawkins, Hicks, Phillips and Moore (1978), and later studies

have specifically tested this hypothesis (De Pablo et al., 1989; De Pablo, Ortiz-Caro, Sánchez-Santed and Guíllamón, 1991). The interpretation in terms of «learned immobility» views immobility as a coping response of the animal, useful to adapt to the situation of the FST. In the first session of the FST the rats learn to be immobile and in the second session they «remember» this immobility (De Pablo et al., 1989). The involvement of learning in the FST has been also pointed out by other authors (e.g., Borsini, Volterra and Meli, 1986; Jefferys and Funder, 1996).

The usual procedure to carry out experiments of FST in mice involves only one six minute session, where the activity in the two first minutes is discarded (Porsolt, 1981). With such procedure it is difficult to study learning. In rats, with two sessions, the behaviour observed in the second session can be compared with that of the first session. In the present study the usual procedure performed with mice had to be modified.

The aim of this study was to test if learned immobility is also present in the FST in mice. Specifically, its main purposes were: a) to study behavioural effects of the repeated exposure to the test that might disclose the existence of underlying non-associative learning, such as habituation, b) to explore if the immobility (supposedly learned) can be forgotten as a consequence of the passage of time (Ebbinghaus, 1913) by means of increasing the time intervals between the two sessions (e.g. Platel and Porsolt, 1982), and c) to test if scopolamine, a muscarinic receptor antagonist, and physostigmine, an anticholinesterasic agent, act respectively as blocker and enhancer of memory consolidation, when given after the first session, always in the FST. In the present experiments, a variation of the original Porsolt test was used, placing the mice in the water tank in two or more occasions separated by diffe-

rent time intervals, and recording swimming activity automatically.

### Methods

#### *Subjects*

All experiments used OF 1 male mice (IFFA CREDO, Lyon, France). The animals were housed in groups of five in standard plastic cages (27 x 27 cm) located in a temperature controlled room ( $21 \pm 2$  °C). Food and water were freely available and a reversed 12 hours light/dark cycle was in effect (lights off: 08:00 hours, local time). The mice were kept a minimum of 4 days after their arrival in the laboratory before testing, and weighed 22-38 g at the time of the experiment. The tests were always carried out during the dark phase of the light cycle.

#### *Apparatus*

A Panlab Animal Activity System (PAS, Panlab, Barcelona, Spain) was used. It consisted of a square platform (35 x 34.5 cm) in the centre of which an upright cylindrical Plexiglas tank (height: 60 cm, diameter: 20 cm) containing 10 cm of water at 25 °C was placed. The equipment was a computerised version of a similar one described in detail elsewhere (De Pablo et al., 1989). In brief, general activity is evaluated as a function of the variations produced by mice swimming activity on the standard frequency of the electromagnetic field of the sensory unit. Frequency variations are transformed into voltage changes, which, in turn, are converted into impulses that are collected by a computer. In Experiment 3 a different platform, provided by the manufacturer with higher sensitivity than that of previous experiments, was used in physostigmine groups. This explains that the average activity values of these groups were approximately twice that of the rest (see Figs. 1a, 2, 3a and 3b).

#### *Drugs*

The following agents were used in this study: scopolamine hydrobromide (Sigma-Aldrich Química, S.A., Madrid, Spain), and physostigmine salicylate (Sigma-Aldrich Química, S.A., Madrid, Spain). Both drugs were dissolved in physiological saline and given i.p. in a volume of 0.01 ml/g body weight. Control group received the same volume of physiological saline. Doses of drugs refer to the salt.

#### *Procedures*

In every experiment the groups were of 15 animals, except for the Sa group of the Experiment 3 that was of 14 subjects. In Experiment 1 the subjects underwent five consecutive sessions of the FST in days 0, 1, 3, 5 and 7. In the rest of the experiments the animals were submitted to two sessions of FST, separated by different intervals of time. In Experiment 2 the intervals explored were: 1, 3, 5, 7, 14, 15, 18, 21 and 24 days. In Experiment 3 the intervals were of 3 (scopolamine groups) or 24 days (physostigmine groups). The mice were individually forced to swim inside the tank, then removed and allowed to dry before returning them to the home cage. The swimming activity was automatically recorded, minute by minute, during each six minute session. Each mouse swam in fresh water to prevent the effect of soiled substance on immobility (Abel and Bilitzke, 1990).

In Experiment 3, subjects were randomly distributed into six groups: Two groups received saline immediately after the first session of the FST (Sa and Sb, for intervals of 3 and 24 days respectively); two groups received scopolamine, 1 or 2 mg/kg, immediately after the first session (SC1 and SC2); two groups received physostigmine, 0.05 or 0.2 mg/kg, immediately after the first session (P1 and P2). Every subject was injected with saline 30 minutes before the first session.

### Analysis

In Experiment 1, data of swimming activity were submitted to analysis of variance (ANOVA), with two «within» factors: Session, with five levels, and Minute, with six levels. In Experiments 2 and 3, the swimming activity of the two sessions was compared by means of two-tailed Student's *t* tests for related samples.

### Results

#### Experiment 1: Effect of repeated exposure to the FST

A statistically significant decrease in the swimming activity was observed throughout the sessions [ $F(4, 56) = 20.67, p < 0.001$ ]. Newman-Keuls post-hoc analysis revealed that the activity was higher in the Session 1 than in the others ( $ps < 0.001$ ), and in the Session 2 vs. Session 4 ( $p < 0.05$ ) (see Fig. 1a). Similar results were obtained for the main effect Minute [ $F(5, 70) = 25.59, p < 0.001$ ], where the Newman-Keuls post-hoc analysis revealed a higher activity in the Minute 1 than in the others ( $ps < 0.001$ ), and in the Minute 2 vs. Minutes 3, 4, 5, and 6 ( $ps < 0.05$ ) (see Fig. 1b). The interaction Session x Minute was significant [ $F(20, 280) = 7.67, p < 0.001$ ]. The changes in activity within each session were less pronounced in the last two sessions.

#### Experiment 2: Effect of different time intervals between the two sessions of the FST

A significant decrease of mobility in the second session with respect to the first one was found in seven of the nine groups. A statistically significant decrease in the activity was observed with intervals of up to 18 days, but not with longer intervals of 21 or 24 days. The intervals between sessions and their respective *t* values were of 1 [ $t(14) = 5.7, p < 0.001$ ], 3 [ $t(14) = 4.10, p < 0.001$ ],

5 [ $t(14) = 2.2, p < 0.05$ ], 7 [ $t(14) = 5.66, p < 0.001$ ], 14 [ $t(14) = 6.63, p < 0.001$ ], 15 [ $t(14) = 5.49, p < 0.001$ ], 18 [ $t(14) = 5.42, p < 0.001$ ], 21 [ $t(14) = 1.10, p > 0.05$ ], and 24 [ $t(14) = 1.63, p > 0.05$ ] days (see Fig. 2).

#### Experiment 3: Effect of 1 or 2 mg/kg of scopolamine and 0.05 or 0.2 mg/kg of physostigmine administered after the first session of the FST

In the scopolamine part of the experiment, a decrease in the swimming activity in the

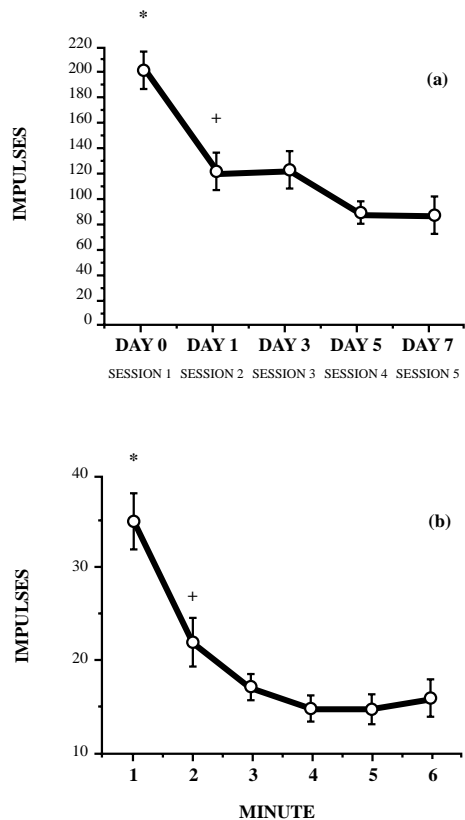


Fig.1. (a) Mean impulses ( $\pm$  SEM) of a group of fifteen mice submitted to five exposures of FST; \* $p < 0.001$  vs. any other session, and + $p < 0.05$  vs. Session 4. (b) Mean impulses ( $\pm$  SEM), minute by minute, of all animals in all days. \* $p < 0.001$  vs. any other minute, and + $p < 0.05$  vs. Minute 3, 4, 5 and 6.

second session with respect to the first one was observed in every group: Sa [ $t(13) = 5.0$ ,  $p < 0.001$ ], SC1 [ $t(14) = 6.6$ ,  $p < 0.001$ ], and SC2 [ $t(14) = 5.6$ ,  $p < 0.001$ ] (see Fig. 3a).

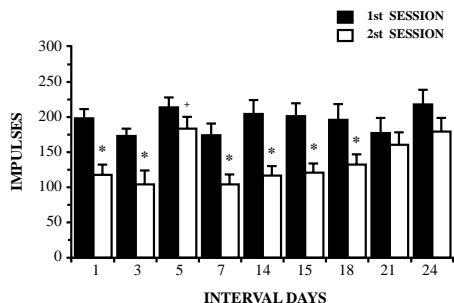


Fig. 2. Mean impulses (+ SEM) of mice tested with intersession intervals of 1, 3, 5, 7, 14, 15, 18, 21, or 24 days. \* $p < 0.01$ , and + $p < 0.05$  vs. first session.

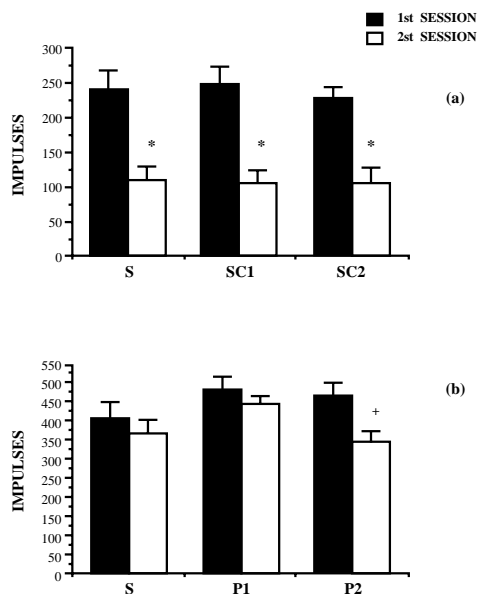


Fig. 3. (a) Mean impulses (+ SEM) of mice tested with an intersession interval of three days. S: Saline; SC1: Scopolamine 1 mg/kg; SC2: Scopolamine 2 mg/kg. (b) Mean impulses (+ SEM) of mice tested with an intersession interval of twenty-four days. S: Saline; P1: Physostigmine 0.05 mg/kg; P2: Physostigmine 0.2 mg/kg. The animals were injected with the indicated substance just after the first session. \* $p < 0.001$  vs. the first session; +  $p < 0.01$  vs. the first session.

With respect to the physostigmine, no significant differences were found in the second session with respect to the first one in the Sb [ $t(14) = 1.26$ ,  $p > 0.05$ ], and the P1 [ $t(14) = 1.05$ ,  $p > 0.05$ ] groups. Nevertheless a significant decrease of the swimming activity was found in the case of the P2 [ $t(14) = 3.17$ ,  $p < 0.01$ ] (see Fig. 3b).

## Discussion

In Experiment 1, where the animals were repeatedly tested in the water tank, one, three, five, and seven days after the first session, a marked decrease in the swimming activity of mice was observed. Such decrease resembles that reported by Kitada, Miyauchi, Satoh and Satoh (1981) in rats: the most striking change was observed from day 1 to day 2. This decrease in the swimming activity can be interpreted as habituation, specially the one observed along sessions (Fig. 1a), since it fulfills the criteria usually ascribed to this phenomenon (Thompson, 1986; Thompson, Donegan and Lavond, 1988). In contrast, the changes observed within a session, along minutes (Fig. 1b), could be attributed to fatigue. The presence of habituation in the FST confirms the involvement of learning in this animal model as previously pointed out by different researchers (De Pablo et al., 1989, 1991; Hawkins et al., 1978). In a review on this subject, West (1990) arrived to the conclusion that the behaviour of immobility in the water cylinder reflects a learned habituation taking place in an environment that has become more familiar to the animal. The influence of «familiarity» to the environment is crucial. In rats, the pre-exposure to the tank without water had a similar effect to that of a tank containing water (Borsini et al., 1986).

If learning and memory processes are involved in the behavioural changes observed in the FST, it may be assumed that forget-

ting of this behaviour must also happen. This was observed in Experiment 2. When the second session took place 21 or 24 days after the first one, the swimming activity in both sessions was similar. These results show that at least a time interval of 21 days between the first and the second sessions must elapse for the immobility behaviour to be forgotten. Nevertheless, intervals of 19 and 20 days have not yet been explored. Unpublished data from our laboratory have replicated the results obtained in Experiment 2 for intervals between sessions of 3, 7, 21 and 24 days at least once, with no contradictory results found at any of the tested intervals. To our knowledge, such strategy has not been used in rats. Platel and Porsolt (1982) showed «forgetting» of exploratory activity in only seven days. Our procedure requires more days but it is also a tolerable time interval in animal experiments.

In the light of the present results and those that point out effects of antidepressants on memory (e.g., Flood and Cherkin, 1987) some recommendations can be made in order to use the FST as a tool for screening substances with antidepressant activity. In the case of evaluating the blocking memory effects, an intersession interval of a few days seems to be appropriate, since in this short period of time there is still no forgetting and there is enough time span to test the animals free of drug. If the drug does have an impairing effect on memory, animals should show as much swimming activity in the second session as in the first one, i.e., no statistically significant differences between sessions should be found. The action of substances that favour memory by preventing forgetting require longer intervals between sessions to be tested. Specifically, the effect of the drug would consist of a diminished swimming activity in spite of the passage of time. The appropriate control for the performance during the second session should be the performance during the first one.

The literature does not reflect any study of the effect of scopolamine, a drug with well known blocking effects on memory, with a procedure of the FST similar to that of the present study in mice. Nevertheless, it has been previously described in rats. This drug, administered i.p. to rats at a dose of 1 mg/kg immediately after the first session, increased the mobility, 24 hours later, in the second session as predicted by the learned immobility hypothesis; nevertheless, the 0.5 mg/kg dose was ineffective (De Pablo et al., 1991). Also, Mancinelli, Borsini, d'Aranno, Lecci and Meli (1988) did not find any effect of scopolamine 0.25, 0.5 or 1.5 mg/kg when given just after the first session. Our results from Experiment 3 agree with those of the last authors showing that scopolamine when injected after the first session had no effect on the second session. We assume that memory consolidation was not affected.

As far as we are aware, not one study has tested the effect of physostigmine with any of the different procedures of the FST in mice. This drug has been tested in a few studies of FST in rats (e.g., Bhattacharya and Sen, 1991; Mancinelli et al., 1988). The procedures of these studies are so different from the present one that any comparison of the results would not be appropriate. Nevertheless, the results obtained are remarkable with respect to the learned immobility hypothesis of the FST. Remember that the interval between sessions was in this case of 24 days. With this period of time between sessions non-treated animals show a similar swimming activity. As was expected, in the control group no significant differences were found between the first and the second session of the test, but there were significant differences between sessions in the group treated with 0.2 mg/kg, showing less activity 24 days later. This result could indicate an improving effect of physostigmine on memory consolidation.

In conclusion, the experiments reported here extend to mice several findings that have been previously made in rats in relation with the FST, and give behavioural and pharmacological support to the learned immobility interpretation of the FST (De Pablo et al., 1989).

### Acknowledgements

Ana Martos and Jorge Parra are thanked for their technical assistance.

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*Aceptado el 30 de octubre de 1998*